User Manual

xHLA

Beta version

2010 (June)

Summary

Introduction

xHLA is a graphical tool based on LabSystem Gen architecture which was developed to assist researchers in pre and post transplant studies in Histocompatibility laboratories. It provides a fast way to researchers manage data of donors and recipients and to study the compatibility between them. The tool has the following features:

1. Graphical and ease to use interface;
2. Data of donors and recipients are stored in a centralized and secure relational database (Access);
3. Compatibility studies use both local and public domain data provided by Web resources.
4. The tool generates a compatibility map in order to assist clinicians in their transplant decisions.
5. Donors and recipient's data stored in other systems or in spreadsheets existing in the laboratory can be used in the compatibility studies easily.
6. Since uses LabSystem Gen architecture, new web resources and algorithms can be added in order to improve tool functionalities.
7. Automation and integration of the HLA Matchmaker and HLA Fusion programs.

We are improving xHLA tool in order to generate more valuable reports to works HLA matchmaker algorithm. These changes are being done every day and new versions will be deployed as soon as possible.

Demo limitations

The demo version has the following limitations:

1. Advanced searchers to local database are not available. These searchers allow researchers to find the best scored potential donors to a specific recipient or, the best recipients to a specific donor. These searchers are performed both in local database and external databases.

2. Data stored in other databases or spreadsheets (external data) can not be integrated to be used in data analyses.

Installing xHLA

The installation procedure is straightforward:

```
------------------------------------------------------------
How to Install
------------------------------------------------------------
1. Download file Application_xHLA.zip
2. File must be placed in directory C. Otherwise, a configuration procedure is
   needed.
3. Unzip file
4. Locate file C:\Application_xHLA\xHLAb.exe
5. Double click xHLA.exe file.
```

Admin user and password (default)

Default user: Admin

Default password: Admin

Downloading NMDP allele code list

The NMDP allele list code must be downloaded every day. In order to download the allele code list, please do the following procedure:

Go to menu **File** and choose the option **Download NMDP Data**. A confirmation dialog box is shown (Figure 1). Choose Yes to confirm operation and the updated data will be downloaded and unzipped. Please, restart application after this operation. Depending on your web link speed, this operation can take several minutes.
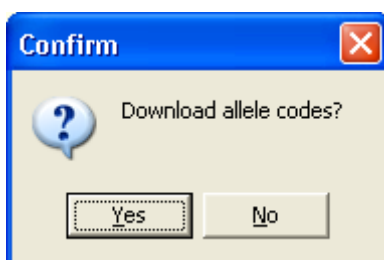
Figure 1 – Operation confirmation before downloading NMDP Data.

Downloading IMGT/HLA data

The IMGT/HLA database can be downloaded every time a new version of the database is deployed. This operation can take several minutes, depending on your web link speed. Please, restart application after this operation.

Go to menu **File** and choose the option **Download IMGT/HLA Data**. A confirmation dialog box is shown **(Figure 1)**. Choose *Yes* to download only missing files. If you choose *No*, the entire database is downloaded and updated locallly.
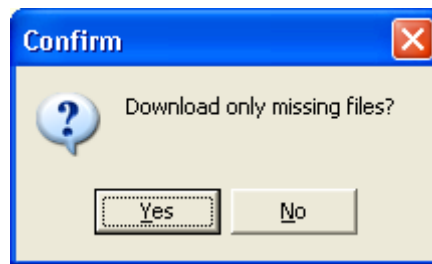

Figure 2 – Choose *NO* to download entire database. Choose *Yes* to download missing files.
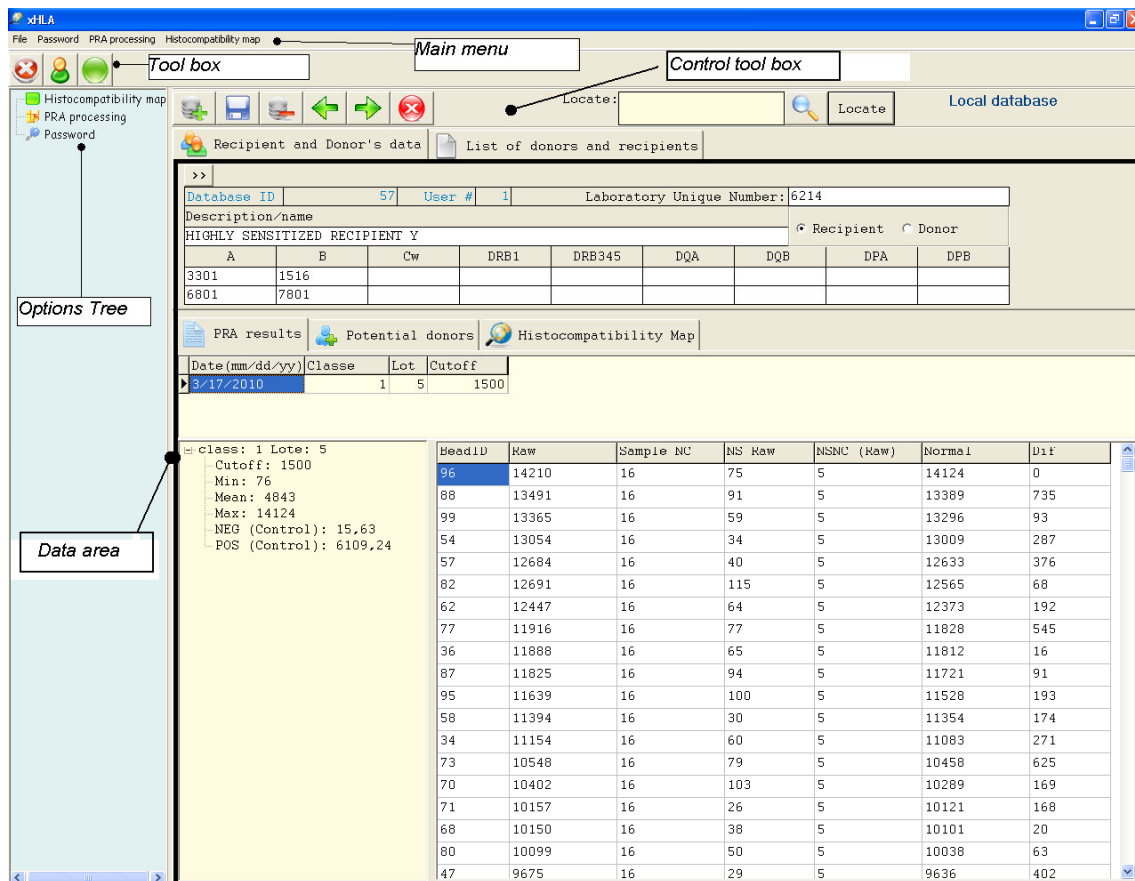
xHLA Main Screen

Figure 3 - xHLA main screen.

The screen is divided in four main areas: i. *Tool box*; ii. *Options Tree*; iii. *Data area* and; iv. *Main menu* area. The *Tool box* is composed of three buttons: Close application; Login/Logoff; and Turn on/ Turn off web link (Figure 4). The green/red ball indicates that a link to web is not available or the link is slow at the moment. To activate the web link, you just click once in this icon and it will become green. The Login/logoff button opens the login dialog box to user lock the station or to log as another user.
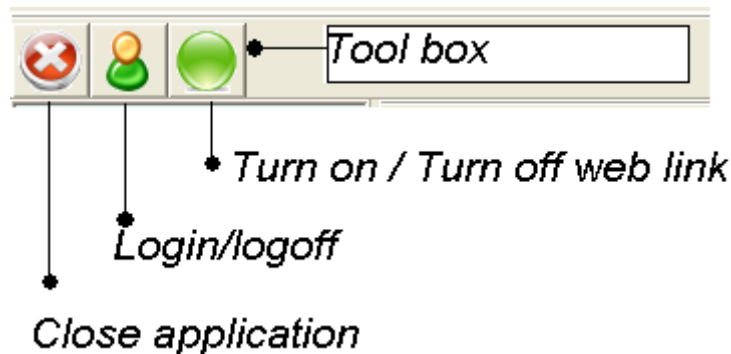

Figure 4 – Tool Box area.

The *Main Menu Area* has the same options showed in *Options* Tree and three additional ones: 1. Exit, Download IMGT/HLA Data and Download NMDP Data (File Menu). These databases are used in generation of the compatibility map. You must download NMDP allele code list every day to have a more accurate analyses.

The *Options Tree* allows user to access the main functions of the application easily. Click over each node existing in the options tree and the Data area (right side) shows the appropriate information.

Options tree

The options tree is a dynamic tree that provides direct access to the System functionalities. There are three main nodes in the tree: **Histocompatibility map**, **PRA processing** and **Password** (Figure 3). Selecting the **Histocompatibility map** node, the *Data Area* shows the form **Local Database** data (donors and recipients) repository and the compatibilities maps generated (detailed later). The **PRA processing** node shows, in *Data Area,* the form **Process files.** Using this form**,** PRA results generated by laboratory's equipments are loaded and processed. The **Password** node *shows* the form **Password** that provides functionalities to change user´s password and to create new users. **Figure 5** shows the **PRA processing** form and form **Local Database** is show in **Figure 3.**

Figure 5 – PRA processing and Password forms.

General Workflow – Processing PRA Results

Read this section carefully before using the xHLA program.

1. **First time using xHLA, select the Input, Output and HLA Matchmaker directories (PRA processing node):**

   a. **Input Directory** – CSV files, containing PRA results, generated by laboratories equipments (Luminex), must be stored in this directory, whose default value is c:\Application_xHLA\Input. If you wish, click "Change…" button to set a new directory path (Figure 5).The PRA files stored in this directory are shown in **Available CSV files** area (Figure 5, on left). *WARNING*: Do not allow or copy any other file format to the Input Directory, because xHLA can fall into an endless loop (buggy).

   b. **Output Directory** – It is used by xHLA as a place to temporary storage while processing data. The default value is c:\Application_xHLA\Output (Figure 5, bottom). Click button "Change…" to set a new directory, if wished.

   c. **HLA Matchmaker Directory** – It is where HLA Matchmaker programs must be stored (Figure 5, bottom). Before copy a version of the HLA Matchmaker program to this directory, be sure that the PANEL sheet was properly filled up and that information about beads/lot were correctly inputted (xHLA only accepts *Omni Lambda*). Once a copy of the HLA Matchmaker program is coppied to this directory, they can be added to xHLA System:

      i. **Addind a new HLA Matchmaker program:** Click "Add..." button to load a new HLA Matchmaker program and follow the sequence showed in Figure 6. During the addind process, xHLA retrieves information existing in HLA Matchmaker spreadsheet to determinate the lot and class (I or II) of the program. However, it is better to set them manually or correct this information if retrieved erroneously, after step 3 of Figure 6. A new program has to be loaded every time version or lot information (PANEL sheet) change.
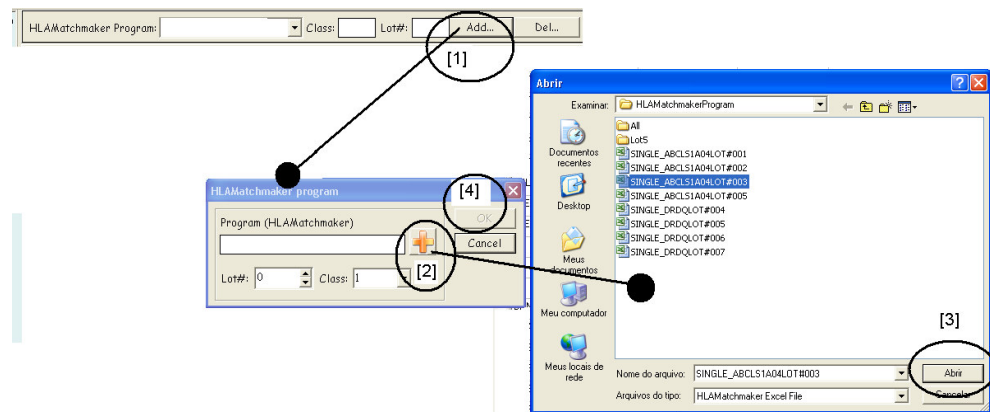
Figure 6 - Adding an HLA Matchmaker program. Follow steps 1 to 4. After clicking button OK ([4]), several minutes can be needed. Please, wait.

    ii. **Deleting a program (**Figure 6**, top):** Click "Del.." button to delete the selected program.

2. Select the appropriate program:
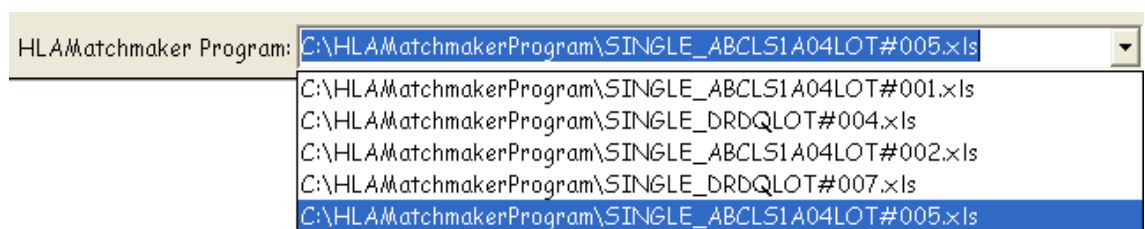
3. Select the desired CSV file generate by LUMINEX equipment with PRA results.
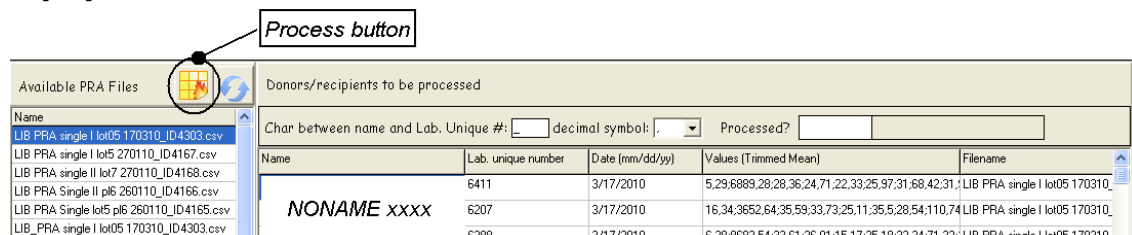
Figure 7 – Processing PRA results.

If the CSV file is a valid one, the data is showed in the matrix showed in Figure 7. Click the *Process button* (on detail) to processe file. Once a CSV file is processed, the PRA results loaded from CSV files are visualized in the form **Local Database** (*Histocompatibility map node*). A record in the local database is added for each recipient processed for the first time.

WARNING: xHLA uses internally a unique number named *Laboratory Unique Number* or LUN (Figure 3, Data Area). This number is used to identify a donor/recipient internally by the System and it is a sequential unique number that is created for each patient (social security number

can be used as LUN, for example). The files containing PRA results must be produced considering this important pre-requisite. The sample description must contain the patient name and this number (one number per patient). The description must set as shown below:

Patient name[specialCaracter]***laboratoryUniqueNumber***

> Where [specialCaracter] can be: #, _, @. Avoid using **;** or **,**. Usage example: *JohnSmith_**9431***

> In LIB laboratory, it is used the underscore (_) as the special character

> If another character is going to be used by Lab, please inform the System in the text edit box named "Char between name and Lab. Unique #" (Figure 7, top).

4. Search the recipient in the Local Database form to check PRA results, after processing operation. To access this form, click the Histocompatibility map node (*Options Tree*) and locate a recipient/donor, as explained in "General Workflow – Generating Histocompatibility Maps" section.

General Workflow – Generating Histocompatibility Maps

A Histocompatibility map can be generated if the donor and recipient's HLA typings are available. However, maps generated to highly sensitized patients require PRA results. To load PRA results related to a patient, see "General Workflow – Processing PRA Results" section. The steps to generate a histocompatibility map are described below:

1. Search the recipient.

a. **Click the *Histocompatibility map* node (Options Tree).**

b. **The form Local Database (**Figure 11**) is shown. There are two main areas: "***Donors and recipient's area***" (**top**) and "***Histocompatibilty study's area***" (**bottom**).**

c. Click a field of the form **Donors and recipient's area.** This field will be considered by the System as parameter in searchers**.** For instance, click the Description/name field if you desire to search for a record using its description information as search parameter.

d. Click the **Locate** text box (on top) and it will become **Search for** text box.

e. Type information in the **Search for** text box that will be used as search parameter. (Example: type JOHN, if Description was selected as parameter).

f. Click the button **Locate** (In the example, all records containing the word JOHN are returned by the System).

g. If one or more records match, they will be presented to User. If more than one is retrieved, click the **list** of **donors and recipients** tab to see all at once. (In the case of the example, JONH something or something JOHN will be retrieved.

2. **Type the HLA typing information in the HLA typing area (**Figure 11**). *Warning*: Some allele codes are not recognized by HLA Matchmaker program and, in these cases, the most frequent in population are used instead. During allele codes typing, the System presents them in green color, if the typed allele is valid. Invalid alleles are shown in black color (**Figure 9**)**
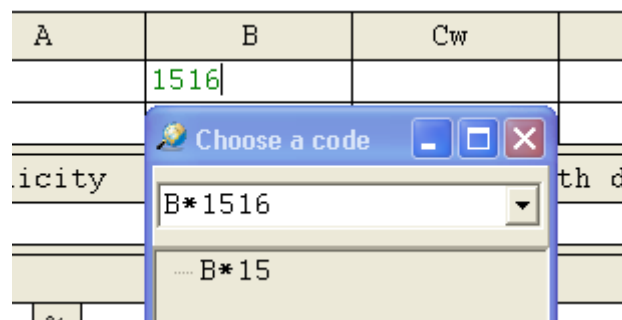
Figure 8 – Typing na allele code. The list of codes is retrieved from IMGT/HLA database.

3. **Define the Cutoff. If PRA results are available to the selected patient, they are presented in the *PRA results tab* (**Figure 9**) in a HLA-Fusion like report.**
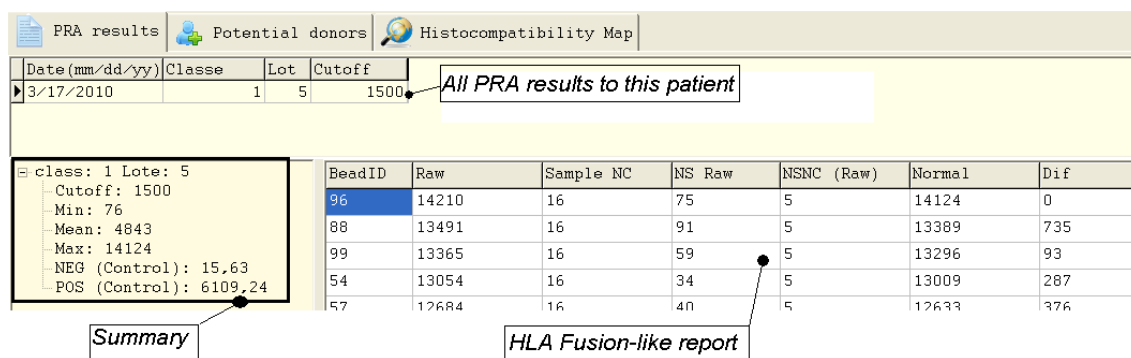
Figure 9 – PRA results of a patient reported to User.

4. If a potential donor is available but not include yet, add him/her into Local Database (see Adding, deleting and navigating and Local Database **section). If he/she is already in database, goto step 5. If no donor is available, go to step 6.**

5. If a potential exists and he/she was stored previously in database, select the "Potential donors tab" **in the** "Histocompatibilty study's area" **(**Figure 10**). Click add button [1], and locate the donor in the dialog window "**Search**". Type the name of the desired donor in the** "Search for" **edit box and press** "Enter"**. The donors retrieved are listed in the matrix [3]. Choose one and click** "OK" **button [4] to add him/her. Click** "Cancel" **button if you decided to abort operation.**
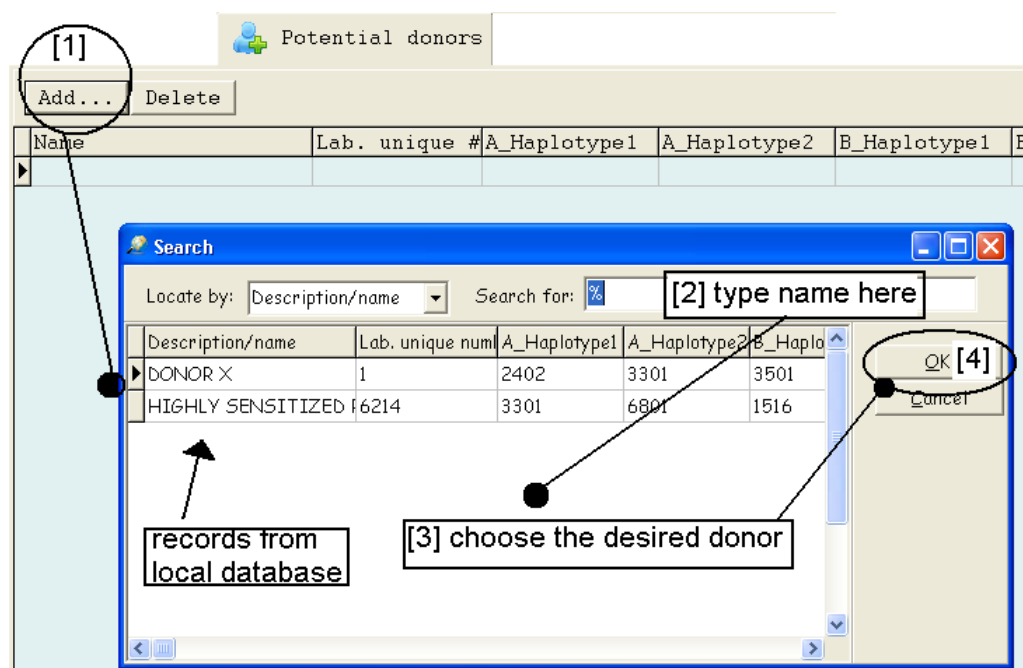
Figure 10 – Steps to add a potential donor to a recipient.
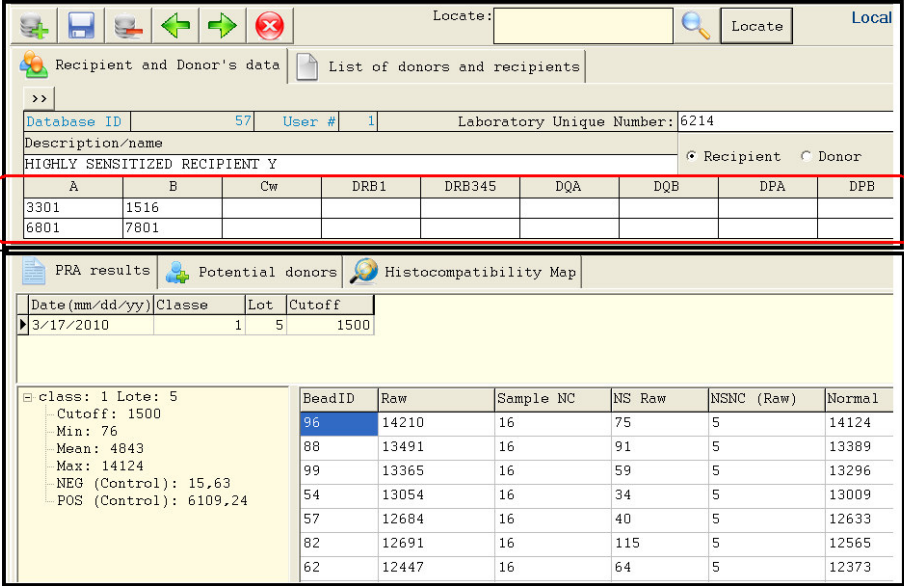
6. Click the "Histocompatibility map" **tab to generate the histocompatibility map.**

Adding, deleting and navigating Local Database

Patient and donor's data are stored in a Local Database (Local repository). Thos section explains how to maintain data stored in it.

Adding:

1. Click the *Histocompatibility map* node (Option trees). The local database form will be available as shown in Figure 11. Click the icon in the tool bar. This will add a new (blank) record.

Figure 11 – Local database form.

2. Type the information of the donor/recipient. The HLA typing is stored in fields A, B, Cw, DRB1 and son on. Haplotype 1 is the upper line and, the haplotype 2 is the lower line. The Description/name field is required.

3. Click the icon to save the information.

Deleting:

Locate the record (see step 1 in *General Workflow – Generating Histocompatibility Maps* section). Click the delete button ⬛.

Navigating:

To navigate**,** use buttons prior and next ⬅➡.

Canceling:

To cancel an input: click cancel button ❌.

Compatibility/Histocompatibility Map

Introduction

xHLA generates a histocompatibility map to assist researchers and clinicians in their pre- and post-transplantations decisions. The map is divided in two major parts and, each part, can be visualized in different level of details (). The data to generate a histocompatibility map is retrieved from several sources, including:

a. Laboratory private data: Luminex, donor and recipient's clinical and genetic data and spreadsheets.

b. Public database/web site data: IMGT/HLA database and NMDP site.

c. HLA Matchmaker program.

One compatibility map is generated for each donor-recipient pair and the recipient can be a highly sensitized one. To illustrate its use, the HLA typing in Figure 12 will be considered to generate a Class I map. The PRA results and any other data needed to simulate this example are available in www.ufpi.br/LIB web site (xHLA programa download).

Recipient	Donor
A*3301	A*2402
A*6801	A*3301
B*1516	B*3501

B*7801	B*7801

Figure 12 – HLAtypings used to generate the Histocompatibility map of Erro! Fonte de referência não encontrada.

The Histocompatibility map

The input data to generate a compatibility map are: The recipient and donor's HLA typings, the PRA results and cutoff. Changing any of these variables, the resulting map also will be different. The map showed in Figure 13 is a complete one because the four inputted variables were provided. If only HLA typings are provided, the Eplets map is not generated, because PRA results are needed to generate it. On the other side, if the donor's HLA typing is not provided, the Alignment map is not generated, because both HLA typings are needed to generate it. The histocompatibility map is the eplet and alignment maps together and, information existing in one, complements the data existing in the other.
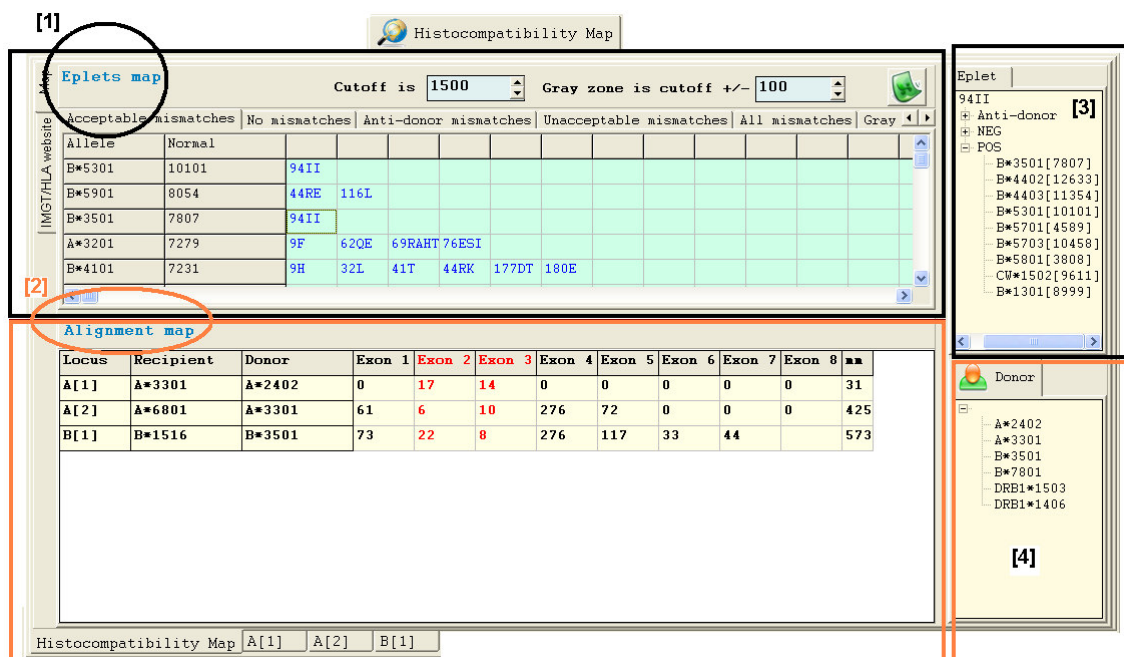
Eplets maps are generated based on HLA Matchmaker program and eplets data and they are important to highly sensitized patients. The Alignment map is generated using data provided by IMGT/HLA database and, its advantage, lays on the fact that PRA results are not needed to generate it.

Alignment map

The alignment map is generated, using the donor and recipient's HLA typings, as follows:

a. For each locus, the allele codes of the donor-recipient pair are compared to each other and, if they don't match, their nucleotide sequences are retrieved from IMGT/HLA database and aligned.

b. The alignment mismatches are counted and summarized in the Alignment map (Figure 13, [2]).

c. Each line of the Alignment map is summary of the alignment performed. The details of this alignment are showed in detail by the *Detailed alignment map*(Figure 14). This detailed map contains three matrixes of data: *Alignment matrix*[1], *Mismatches matrix* [2] and *Number of mismatches by exon matrix* [3].
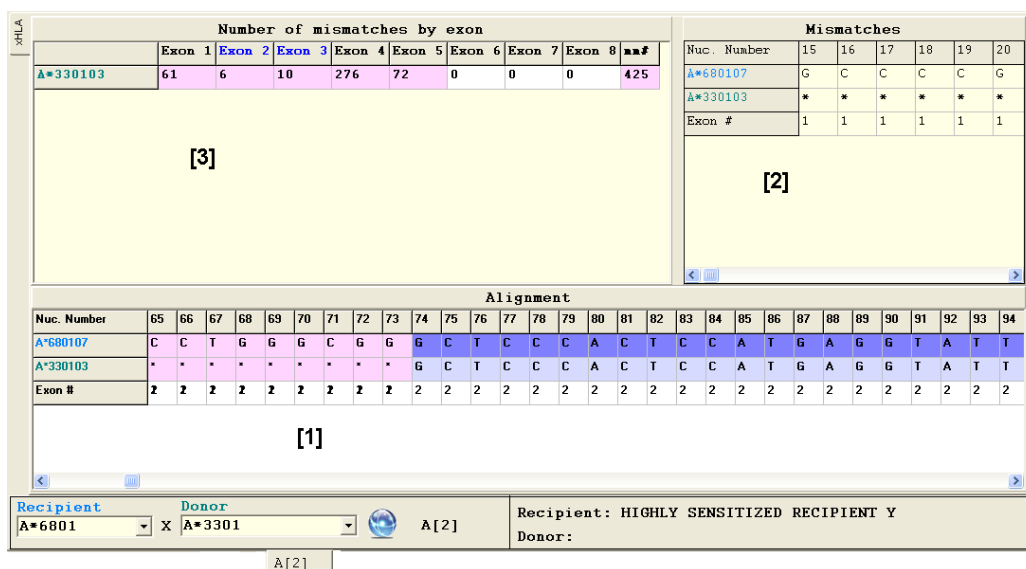
Figure 14 – Details of the alignment between A*6801 and A*3301.

The Alignment matrix shows the alignment of the nucleotide sequences retrieved from IMGT/HLA. This matrix has four or more lines: Nucleotide position, recipient's allele code, one or more donor's allele codes and the Exon number. If one position is pink colored, it means that there is a mismatch in that position. If an asterisk is showed in one position, it means that the nucleotide is unknown in that place and they both are counted as mismatches. The *Mismatches matrix* shows the mismatching nucleotides found during alignment process and, the *Number of mismatches by exon* (NME) *matrix* summarizes the Mismatches matrix, exon by exon. The Aligment Map showed in Figure 13 is the union of all *Number of mismatches by exon matrixes* generated to the donor-recipient pair.

The generation of the Alignment map using NMDP codes as input is also possible, however the xHLA System needs to retrieve allele list codes from the NMDP web site and combine them. The result is an Alignment map showing all alignments possible. Figure 15 is an example. The donor's A*01CD creates two lines in the Alignment matrix because the allele list codes returned to this NMDP code are A*0103 and A*0104. So, the A*2633 (recipient) is aligned to each allele of the list.
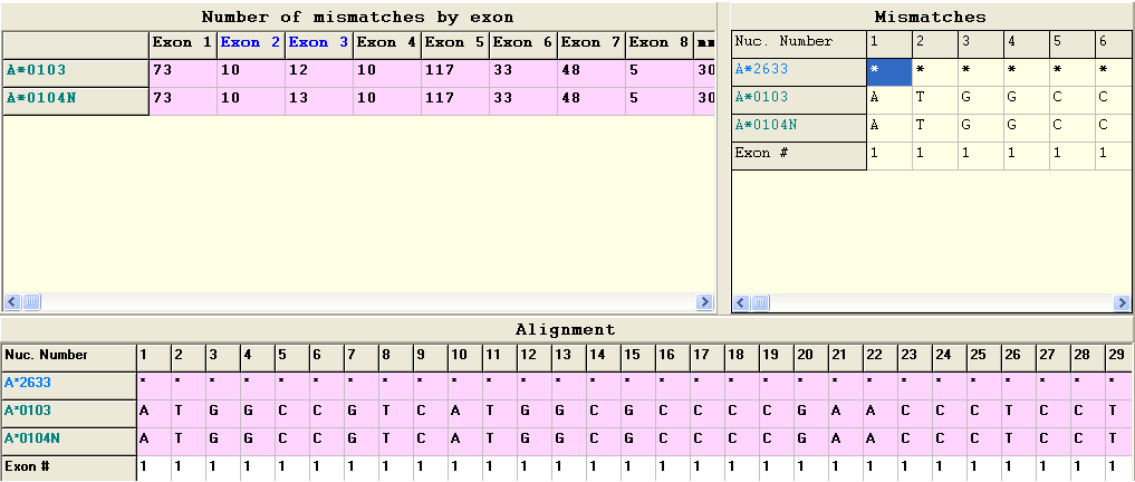
Figure 15 – Alignment details usign A*2633 and A*01CD (NMDP code). The allele code list returned to A*0CD is (A*0103 and A*0104N).

The alignment in the IMGT/HLA website can also be visualized on line easily, just clicking the button . This operation executes an integrated browser as show in Figure 16. Notice that xHLA uses old nomenclature to generate maps, but since nomenclature has changed in april, during web sites queries, they are

translated to new nomenclature. The xHLA system still uses the old nomenclature because HLA MAtchmaker program was not updated until now.
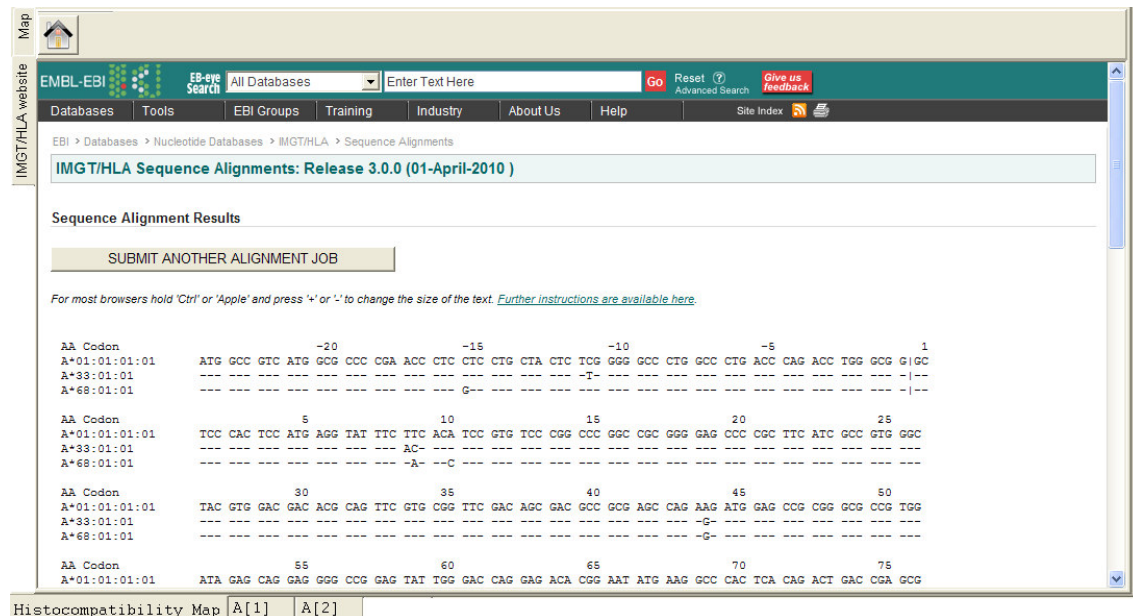
Figure 16 – IMGT/HLA website integrated to xHLA graphical interface.

The best Alignment map shows the columns exon 2 and exon 3 with zero mismatches because clinically has been reported that mismatches in these two exons increase the chances of graft failures. During searches in waitlists or databases, the xHLA System tries to find a donor-recipient pair with no mismatches in these two exons.

Eplets map

To generate an Eplet map, the minimum inputted data are: Recipient's HLA typing, PRA results (Luminex csv files) and the eplets for each HLA molecule. In molecular terms, a particular HLA molecule may present non-linear sequences of amino acids (eplets) potentially shared by other HLA molecules. HLA molecules that share the same eplets, for which the patient has preformed antibodies and can be potentially recognized, contraindicate transplantation. On the other hand, eplets that are not recognized by preformed antibodies in the renal transplant recipient, in theory, offer no danger to the transplant. As a result, any HLA molecule consisting of only eplets not recognized by the antibodies of the patient are acceptable for transplantation. Such molecules are

now known as HLA acceptable mismatches. The elucidation of involved eplets can be achieved by running the HLA Matchmaker (HMM) algorithm and, the xHLA generates the Eplets map based on outputs generated by this program.

The eplets map generation in based on Cutoff value. These values will determinate which HLA molecules are positively recognized and negatively recognized by antibodies. So, the cutoff generates two main sets of data in the PRA results: POS and NEG. The determination of the cutoff is crucial in results and, the xHLA produces a report, basead on PRA results, to assist the researcher in this determination (Figure 11, "Histocompatibility Studys are"). The report shows the PRA results ordered from highest value to lowest value. The idea is to try to determinate in which moment the Normal value decreases rapidly (mostly cases). In the example, the cutoff was determinate as 1500 (Figure 17). Sometimes, the cutoff cannot be easily determinate and the default initial value becomes 500.

Figure 17 –The chosen cutoff value was 1500 because the higher Dif value encountered was 854 (beadID 17 and 63).

All values above Cutoff is added to the POS set and, all values below cutoff, including itself is added to the NEG set. An additional set, name GRAY ZONE is also created. This set is the PRA results whose normal values are too close to the chosen cutoff value (up or down). xHLA uses these two sets to create the following five Eplets sets:

Table 1 – Eplets sets provided by Eplet Map.

1. Acceptable mismatches	No pre-formed antibody against these HLA molecules'
2. Unacceptable mismatches	There are pre-formed antibodies against these HLA molecules

3. Gray Zone mismatches	These molecules are too close to cutoff
4. No mismatches	These molecules have the same eplets if compared to recipient's HLA typing molecules.
5. Anti-donor mismatches	If a donor was provided, the differences, between donor and recipient are presented here.
6. All eplets	Union of the POS and NEG sets.

The "blue eplets" algorithm – Acceptable and unacceptable mismatches.

If an eplet is in the NEG set, it means that pre-antibodies against it were not found. The "blue eplets algorithm" paints in blue color all eplets found both in NEG and POS sets. If all eplets of a HLA molecule in POS set becomes blue, it means that no antibody against this molecule has been found and it is an acceptable mismatch (Figure 18, [1]). After executing this algorithm, new two sets are created: The acceptable and unacceptable mismatches sets. The first one contains all molecules for whose there are no pre-formed antibody and, the second one contains all HLA molecules for whose there are pre-formed antibodies against then. Shortly, the acceptable mismatches are the **NEG set** + molecules of the POS set whose all eplets are in **NEG set** too. The unacceptable mismatches are the **POS set** - blue molecules of the POS set. After executing this algorithm, the cutoff can be or not be upgraded. See in Figure 18 that if, the eplets 44KM and 152RW were in NEG set also, the cutoff would be upgraded to 2762. However, because they are not in NEG set, the cutoff remains 1500 (44KM and 152RW are positives).
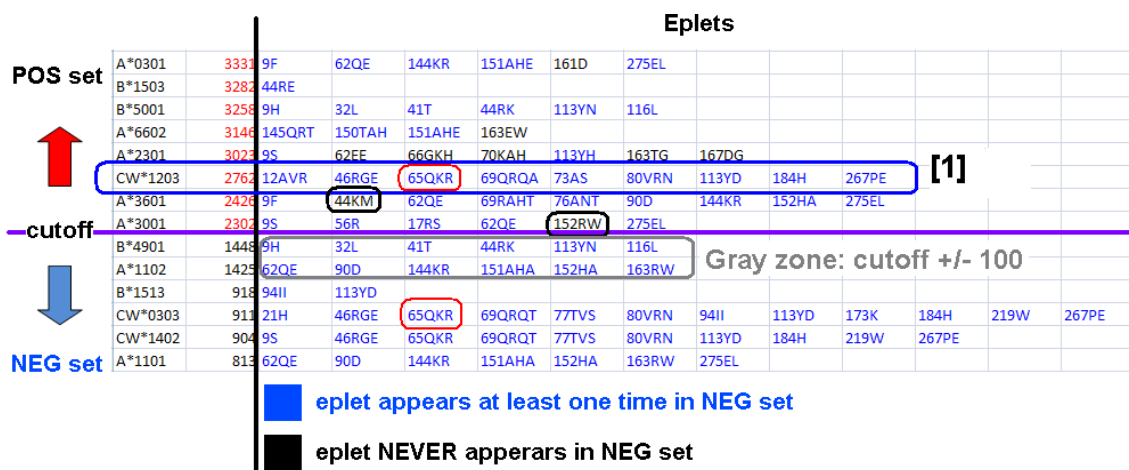
Eplets

A*0301	3331	9F	62QE	144KR	151AHE	161D	275EL						
B*1503	3282	44RE											
B*5001	3258	9H	32L	41T	44RK	113YN	116L						
A*6602	3146	145QRT	150TAH	151AHE	163EW								
A*2301	3023	9S	62EE	66GKH	70KAH	113YH	163TG	167DG					
CW*1203	2762	12AVR	46RGE	65QKR	69QRQA	73AS	80VRN	113YD	184H	267PE			[1]
A*3601	2426	9F	44KM	62QE	69RAHT	76ANT	90D	144KR	152HA	275EL			
A*3001	2302	9S	56R	17RS	62QE	152RW	275EL						
B*4901	1448	9H	32L	41T	44RK	113YN	116L						
A*1102	1425	62QE	90D	144KR	151AHA	152HA	163RW						
B*1513	918	94II	113YD										
CW*0303	911	21H	46RGE	65QKR	69QRQT	77TVS	80VRN	94II	113YD	173K	184H	219W	267PE
CW*1402	904	9S	46RGE	65QKR	69QRQT	77TVS	80VRN	113YD	184H	219W	267PE		
A*1101	813	62QE	90D	144KR	151AHA	152HA	163RW	275EL					

POS set ↑ — cutoff — Gray zone: cutoff +/- 100 — ↓ NEG set

■ eplet appears at least one time in NEG set

■ eplet NEVER apperars in NEG set

Figure 18 – The "dance of eplets" algorithm. 65QKR was found both in NEG and POS sets.

Another important analysis is to see if the eplet in both POS set and NEG set appears only in gray zone. The gray zone is determinate by user and is a values around cutoff value. By default, xHLA determinates the gray zone as Cutoff value +/- 100. In the example, gray zone ranges from 1400 to 1600 (normal value). Eplets found as neg and pos in this zone can be a false positive ou false negative. So, it is better not to find the eplet only in gray zone. Farther from the Cutoff is found na eplet, more positive or negative is the eplet.

Eplet report

In order to facilitate compatibility analyses, once the User clicks an eplet, a report to it is generated and showed as seen in Figure 19. The eplet 32L was found in NEG set, POS set and Gray zone set. Although it is an acceptable mismatch, once it is both in NEG and POS sets, it can be a false negative because this eplet is only in the negative part of the Gray zone. The eplet 62GE is only in the POS set and, additionally, it was found in three molecules as the possible unique positive eplet, including in the molecule B*5801 (very far from cutoff). In this case, there is a high positivity associated with this eplet. The 70KHA is similar to 62GE, however it was found in the donor HLA typing. On

the other side, the 44RE has a high negativity associated with this eplet because it was found in HLA molecules with normal values to far from cutoff.
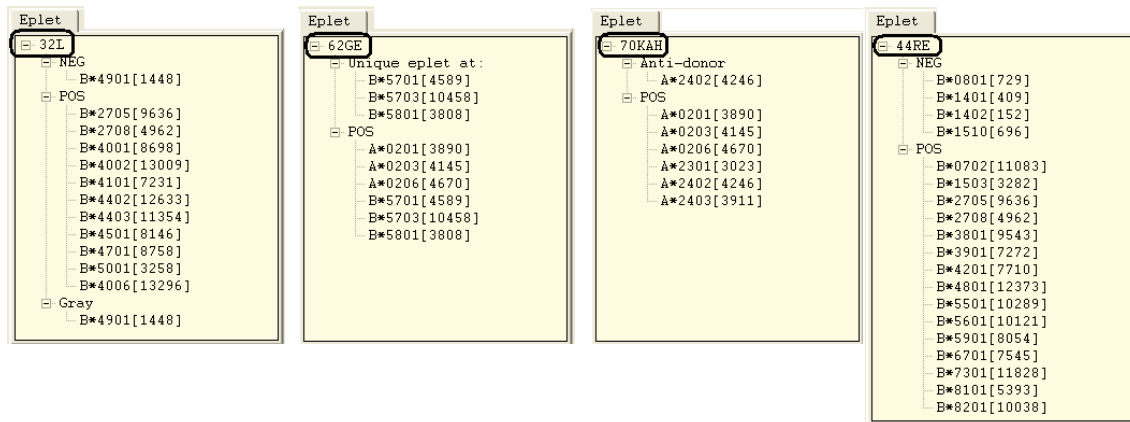

Figure 19 – Three diferent eplets and their reports. The report gives a more accurate vision about the selected eplet.

Histocompatiblity maps and searches in databases

The xHLA program can use the sets acceptable and unacceptable eplets to perform searchers in databases in order to find the best/worst matching to a donor or recipient. This facility can increase the reliability and the chances of highly sensitizes patients in the waitlist to find an organ.

Exporting the eplets map

The eplets map can be exported to MS Excel easily, in order to facilitate the medical reports. In this case, choose one of the eplets sets (Table 1) and click the button ![icon] to export it.

xHLA and the LabSystem Gen Architeture

The xHLA tool uses the architecture provided by LabSystem Gen to perform its tasks. The LabSystem Gen provides tools and a framework that allow researchers to build a Laboratory Information Manager System (LIMS) without programming efforts. The architecture of the System, as it is shown in Figure 20, provides three layers: Application layer, Data access layer and Data storage layer. On the top of the architecture is the Application layer (xHLA is in this layer). The first step towards the creation of a LIMS is to design the Data and Application models using the Designer tool. The Data Model includes a domain-specific data model describing the entities (tables) and relationships (associations between tables) that the researcher wants to manage. The application model, in turn, describes properties that allow the researcher to customize the look and feel of the LIMS graphical user interface (GUI). As show in Figure 20, on top, a Data and Application models can be created based on an experimental plan using the modeling tools provided by Designer. In laboratories. where data are stored in spreadsheets or legacy systems, an automatic approach, using reverse engineering is also possible. In this process, they are used as input to generate the Data model. However, the Application model is still defined using the Designer modeling tools.
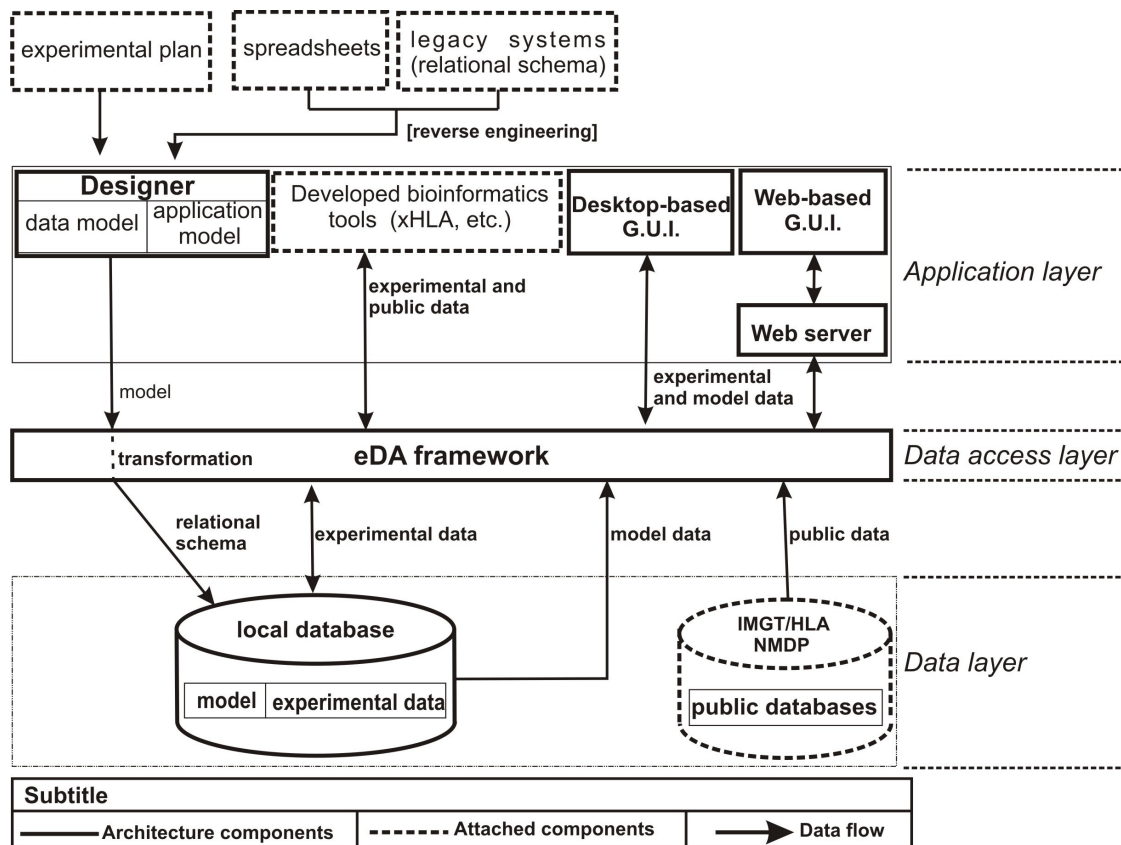
Figure 20 - LabSystem Gen's general architecture.

In the middle layer is the eDA framework. The eDA framework provides transparent access to data stored in databases and performs three major tasks to Application layer: 1. It retrieves data stored in public HLA databases on web. These data can be used in complex analysis performed by bioinformatics tools developed in LIB; 2. It provides functions to store and to retrieve experimental data stored in local relational database and; 3. It interprets a designer's data model and generates a relational schema in the local database (transformation). This generated relational schema stores a copy of the Designer's model for future reference and the experimental data.

Public HLA databases and the local database are in the Data layer. The local database is a backend MySQL relational database provided by System that can be accessed by applications through the eDAframework. Experimental data can be collected and managed using the Graphical User Interface (G.U.I.). The desktop-based GUI is a generic program that dynamically build forms for users

manage data, based on the look and feel specified in the Application model. The web-based GUI, is a set of web pages created dynamically by the Web Server Application and, as desktop-based G.U.I. forms, they are based on Application model and are used for users manage data.

xHLA, as shown in Figure 20, uses the eDA framework functionalities to manage data in its local relational database and to access data existing in web resources.

Report BUGS

Please, any bug report to claudio.demes@ufpi.edu.br.

Manual revision

Unfortunately this manual was not revised until 05/05/2010. Any comments about errors and grammar, please help us to improve it reporting to us.